I-015: Molecular Observations of Anaerobes in Atmospheric Oxygen

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ABSTRACT

A long-standing desire in microbiology is to be able to observe *in situ* and at a molecular level how anaerobes respond to atmospheric oxygen. Over the past decade, physics, engineering and instrumentation innovations have led to the introduction of synchrotron radiation-based infrared spectromicroscopy. Spatial resolutions of less than ten micrometers and photon energies of less than an electron volt make synchrotron infrared spectromicroscopy non-invasive and useful for following the course of cellular processes. Here we present a comparative study of molecular changes in the obligate anaerobe *Desulfovibrio vulgaris* Hildenborough and the facultative anaerobe *Shewanella oneidensis* during their exposure to atmospheric oxygen. Using non-invasive synchrotron radiation-based Fourier transform infrared (SR-based FTIR) spectromicroscopy, we successfully measured directly molecular changes in cellular environments in *D. vulgaris* and in *S. oneidensis* during their exposure to air. By comparing measurements, we were able to identify the time-dependent molecular changes in lipids, nucleic acids, proteins, and polyglucose. Images from fluorescence and electron microscopies provide direct visual images of the corresponding morphological changes.

In this poster we present preliminary results with a primary focus on the short term time-dependent changes in cell lipids, nucleic acids, proteins, and polyglucose molecules.

OBJECTIVES

1. To compare time-dependent molecular changes in the cellular environments of *D. vulgaris* and *S. oneidensis* during their exposure to air using non-invasive synchrotron radiation-based Fourier transform infrared (SR-based FTIR) spectromicroscopy.
2. To gain insight into these molecular changes using images from fluorescence and electron microscopy to provide direct visual images of the corresponding morphological changes.

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MATERIALS and METHODS

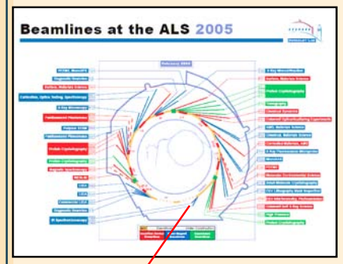
Bacteria strains, culture, and growth: *Desulfovibrio vulgaris* Hildenborough ATCC® Number: 29579 and *Shewanella oneidensis* ATCC® Number: 700550™ were used in this study. The *S. oneidensis* and *D. vulgaris* Hildenborough cells were grown anaerobically in an anaerobic glovebox incubator at 30°C on yeast-free LS4D agar plates containing 50 mM sulfate, 60 mM lactate, Thauers vitamins, trace minerals #3, 0.1 g/L $Fe(NH_4)_2(SO_4)_2$ and PIPES.

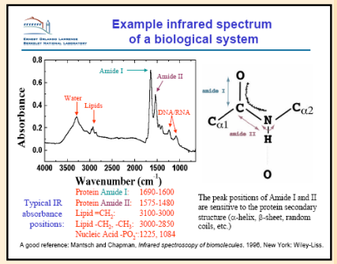
Colonies imprinting and maintenance: Colonies that were formed and grown to about 1 mm in diameter were imprinted onto functionalized gold-coated microscope slides for SR-FTIR or onto glass microscope slides for the membrane integrity stain. They were maintained on the slides at 100% relative humidity and 20°C for 24 hours (SR-FTIR) or 1 hour (for membrane integrity assay) before the oxidative stress experiments.

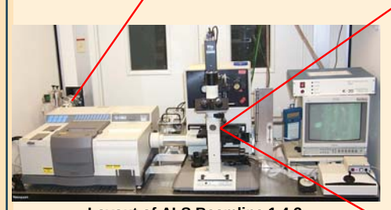
Oxidative stress experiments: *D. vulgaris* Hildenborough on gold-coated slides were transferred anaerobically to the microscope stage incubator and infrared spectral reading allowed to reach steady state. We then recorded time course of infrared absorption intensity, which were indicative of intracellular chemical conditions in different biologically important molecules in *D. vulgaris* before and after exposure to atmospheric oxygen at 100% relative humidity and 20°C for 1.5 hours. Similarly, *S. oneidensis* were transferred to the microscope stage incubator under anaerobic conditions. We again recorded time course of infrared absorption intensity, which were indicative of intracellular chemical conditions in different biologically important molecules of *S. oneidensis* cells during their exposure to atmospheric oxygen also at 100% relative humidity and 20°C for 1.5 hours.

Synchrotron-Radiation based Fourier Transform Infrared (SR-FTIR) spectromicroscopy

A non-invasive analytical tool that can track the progression of biological and biogeochemical processes at a diffraction-limited spatial resolution finer than 10 μm without fixing, staining or labeling cells.







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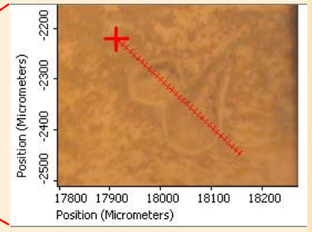


Membrane integrity and cell viability using the Live/Dead *Bac*light™ stain

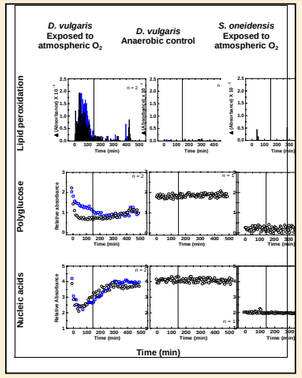
Samples of cells from oxidative stress experiments were also treated with SYTO 9 and Propidium Iodide dyes from the LIVE/DEAD *Bac*Light Kit L-7007 for detection and visualization of their membrane integrity and viability.

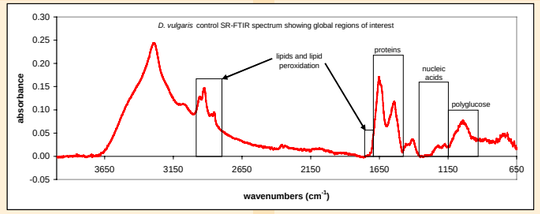
Transmission Electron Microscopy (TEM)

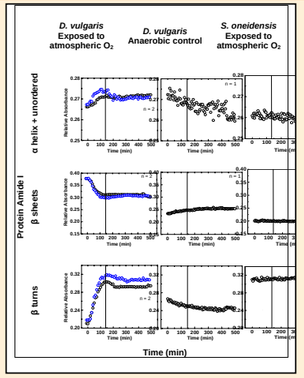
Cell suspensions were pelleted, then glutaraldehyde fixed and post-fixed with osmium tetroxide before staining with uranyl acetate and dehydrating with a series of increasing concentrations of acetone. The dehydrated cell pellets were infiltrated with Epon resin and embedded in pure Epon resin. Ultrathin sections stained with lead citrate and uranyl acetate were imaged using a Tecnai transmission electron microscope operating at 100 kV.

PRELIMINARY RESULTS

S. oneidensis and *D. vulgaris* SR-FTIR



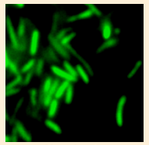




Compared to the anaerobic control and the facultative anaerobe *S. oneidensis*:

- Lipids in *D. vulgaris* cells undergo lipid peroxidation on air exposure, a sign of oxidative stress.
- Air-exposed *D. vulgaris* cells experience a relative drop in polyglucose and nucleic acids.
- Amide I protein within the cell exhibit a complex response suggesting changes in the secondary structure of the proteins

S. oneidensis membrane integrity stain



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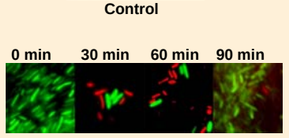
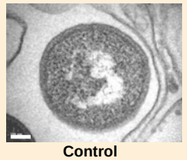


- Cells with undamaged membranes stain green and those with damaged membranes stain red.
- Some *S. oneidensis* cells show membrane damage after about 30 min air exposure.
- Most cells retain membrane integrity after 90 min air exposure.

S. oneidensis TEM



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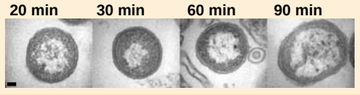
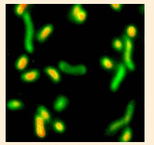


- Some cells show membrane damage after 30 min air exposure.
- There is no obvious visible change in morphology or internal structure as a result of air exposure.

All scale bars approx. 100 nm

D. vulgaris membrane integrity stain



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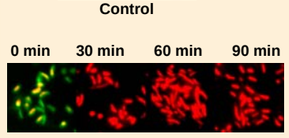
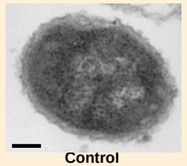


- Cells with undamaged membranes stain green and those with damaged membranes stain red.
- Membrane damage is evident after about 30 min air exposure.
- Some *D. vulgaris* cells recover membrane integrity after 120 min (2 hr) of exposure to air

D. vulgaris TEM



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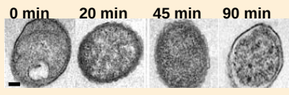

- Some cells show membrane damage after 30 min air exposure.
- After about 90 min of air exposure there is an increase in the width and decrease electron density of the material in the periplasmic space.

REFERENCES

Holman, H.-Y. N., M.C. Martin, and W.R. McKinney (2003). Tracking chemical changes in a live cell: Biomedical Applications of SR-FTIR Spectromicroscopy, in Special issue: First International Conference on Biomedical Spectroscopy: From Molecules to Men, *Spectroscopy - An International Journal* **17**(2-3): 139-160.

Holman, H-Y. N., K. A. Bjornstad, M. P. McNamara, M. C. Martin, W. R. McKinney and E. A. Blakely (2002). Synchrotron infrared spectromicroscopy as a novel bioanalytical microprobe for individual living cells: cytotoxicity considerations. *Journal of Biomedical Optics* **7**(3): 417-424.

Holman, H.-Y. N., M.C. Martin, E.A. Blakely, K. Bjornstad, W.R. McKinney (2000). IR spectroscopic characteristics of cell cycle and cell death probed by synchrotron radiation based Fourier transform IR spectromicroscopy. *Biopolymers* **57**(6): 329-335.

ACKNOWLEDGEMENTS

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